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FREQUENCY OF PANCREATIC BETA-CELL AUTOIMMUNITY MARKERS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

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Abstract A total of 305 ambulatory patients recruited at the Division of Endocrinology, Hospital de Clínicas, University of Buenos Aires, with autoimmune thyroid disease (AITD) were studied to search for associations between autoimmune thyroid disease and presence of serum markers of autoimmune diabetes mellitus. Screening for markers of pancreatic beta-cell autoimmunity was performed by radioligand binding assays (RBA) as follows: autoantibodies to glutamic acid decarboxylase (GADA) and proinsulin (PAA) were determined in all sera, whereas autoantibodies to protein tyrosine phosphatase (IA-2A) and insulin (IAA) were additionally measured in 200 sera randomly selected from the total collection. In addition, every GADA positive serum among the remaining 105 sera was systematically tested for the presence of IA-2A and IAA. In the cohort of 305 AITD patients 22 (7.2%) were previously diagnosed as type 1, type 2 or insulin-requiring type 2 diabetics. Ten of these patients presented serum marker positivity specific for β-cell autoantigens and 12 were marker negative. On the other hand, considering the majority of non-diabetic AITD patients (n=283), β-cell marker positivity was detected in 17 individuals (6.0%). The prevalence of autoimmune diabetes markers was much higher in the studied population than in the general population utilized as a control group, and GADA was the most frequent marker.

Key words: thyroid disease, diabetes, immunological markers, autoimmunity

Resumen ***Frecuencia de marcadores de autoinmunidad beta pancreática en pacientes con enfermedad tiroidea autoinmune***. Se investigó la asociación entre enfermedad tiroidea autoinmune y la presencia de marcadores séricos de diabetes mellitus en 305 pacientes ambulatorios con enfermedad tiroidea autoinmune reclutados en la División Endocrinología. La búsqueda de marcadores de autoinmunidad contra las células beta pancreáticas se realizó por la técnica de unión de radioligandos (RBA) como se detalla a continuación: se determinaron autoanticuerpos contra la decarboxilasa del ácido glutámico (GADA) y proinsulina (PAA) en todos los sueros, mientras que los anticuerpos contra la proteína tirosina fosfatasa (IA-2A) e insulina (IAA) fueron medidos en 200 de estos sueros tomados al azar de la colección total. Además, en los restantes 105 pacientes, la presencia de IA-2A y IAA fue evaluada en todos los sueros positivos para GADA. Del grupo de 305 pacientes con enfermedad tiroidea autoinmune 22 (7.2%) fueron diagnosticados previamente como diabéticos tipo 1, tipo 2 o tipo 2 insulino-requirientes. Diez de ellos presentaron positividad para marcadores específicos de autoantígenos de célula β, en tanto 12 fueron negativos. Por otra parte, en 17 de los 283 pacientes (6.0%) con enfermedad tiroidea autoimmune y sin diagnóstico previo de diabetes, se detectó positividad para marcadores de célula β. La prevalencia de marcadores de autoinmunidad asociados a diabetes fue mayor en la población estudiada que en la población general usada como grupo control, siendo GADA el marcador más frecuente.

Palabras clave: enfermedad tiroidea, diabetes, marcadores inmunológicos, autoinmunidad

Autoimmune endocrine diseases are disorders in which immune dysregulation results in certain organ-specific aggression. Many autoimmune diseases are chronic conditions that progress over the course of years and are characterized by the presence of autoantibodies that precede the overt disease by months or years. While these immune disorders can affect many different glands, the most common disorders are, by far, autoimmune thyroid disease (AITD) and type 1 diabetes mellitus (T1DM). Both diseases share similar HLA association and pathogenesis, which involve T-cell infiltration resulting in dysfunction of the target organ. A significant association between AITD and T1DM has been previously shown[1]. More than 20% of patients with T1DM have thyroid antibodies and

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50% of them progress to clinical AITD[2]. Conversely, in some patients initially presenting isolated AITD, symptoms of insulin-requiring diabetes appear after a variable period of time[3]. At present, however, the association between autoimmunity and diabetes is under revision. In fact, diabetes mellitus can be considered as a heterogeneous group of hyperglycaemic diseases in which the most frequent forms are type 2 diabetes mellitus (T2DM) and T1DM. The most prevalent variant, T2DM, occurs in adult life and is due to insulin resistance and/or inadequate compensatory insulin secretion, generally without evidence of immunological aggression[4]. The other variant, T1DM, which afflicts mainly children and young patients, was classically considered the only one to be associated with specific autoimmune disturbance. However, in some instances (e.g. certain adult-onset patients having autoimmunity and/or insulin dependence) it is difficult to differentiate between the two variants[5]. To solve these discordances with the classical types, some investigators have proposed new categories such as marker positive, insulin-requiring type 2 diabetes mellitus (IR-T2DM)[6] or latent autoimmune diabetes of adult (LADA)[7, 8]. In addition, according to recent work of our laboratory, autoimmunity markers profile in adult-diagnosed diabetes varies according to the time elapsed between diagnosis and insulin dependence[9].

Autoantibodies may also predict specific clinical manifestation, disease severity, and rate of progression. The identification of these markers and the assessment of their predictive value might enable secondary prevention using specific drugs or by immunological treatment[10]. In addition, the ability to predict the severity of disease and its specific clinical manifestation allows tertiary prevention of disease complications. Such prevention may be accomplished by relatively simple adjustments of therapy and lifestyle.

The aim of the present work was to investigate associations between autoimmune thyroid diseases and autoimmune diabetes in an Argentinean population sample. Accordingly, we measured autoimmune diabetes markers in a large group of patients with autoimmune thyroid illnesses referred to the Division of Endocrinology, Hospital de Clínicas, University of Buenos Aires.

Materials and Methods

Blood samples from 305 ambulatory patients with autoimmune thyroid disorders were collected after an overnight fast, and sera were stored at –20 °C until assayed. Diagnostic classification of the thyroid abnormality was done according to routine clinical, imaging, and biochemical data, including thyroid peroxidase and thyroglobulin autoantibodies assessment (TPO-Ab and TG-Ab, respectively), beside the classical hormonal profile. Out of the 305 patients, 73 (23.9%) had Graves' and 232 (76.1%) Hashimoto's disease. Diagnosis of diabetes was performed according to the American Diabetes Association[11]. Clinical classification, gender, age, and previous history of diabetes are summarized in Table 1.

Control sera from 100 healthy individuals with no personal or family history of diabetes or other autoimmune diseases were obtained from blood donors at the Blood Bank of the hospital. The investigation was performed according to a protocol approved by the institutional review committee and written informed consent was obtained from all patients.

Marker screening was performed as follows: autoantibodies to glutamic acid decarboxylase (GADA) and to proinsulin (PAA) were determined in all samples, whereas autoantibodies to protein tyrosine phosphatase (IA-2A) and insulin (IAA) were additionally measured in 200 randomly selected sera. Every GADA positive serum among the remaining 105 sera was also systematically tested for the presence of IA-2A and IAA.

GADA determinations were performed as detailed elsewhere[12, 13]. Briefly, GADA was measured by radioligand binding assays (RBA) using ^{35}S-labeled GAD65 expressed *in vitro* in a reticulocyte lysate system (Promega, Madison, WI). Size of the labeled product was checked by SDS-PAGE fluorography.

IA-2A was determined by RBA, essentially as described for GADA, with IA-2 obtained using the IA-2ic gene coding for residues 604-979 of IA-2[14].

IAA was assessed according to the original procedure of Palmer et al.[15] and performed essentially as described by Stumpo et al.[16]. Each sample was assayed in duplicate and the non-specific binding was determined, in parallel, using an excess (7.5 µM) of unlabeled human insulin. PAA was assessed by using ^{125}I-Proinsulin as a tracer following a similar protocol. The specific insulin or proinsulin binding percentage (sB%) was calculated as the difference between B% from samples incubated with or without cold hormone excess.

The performance of RBA procedures for GADA, IA-2A and IAA was controlled externally in international proficiency tests

TABLE 1.– *Clinical classification of patients under study*

	n	M/F	Mean age (years)	Total n (%)	Diabetic patients T1DM n (%)	T2DM n (%)	IR-T2DM n (%)
AITD	305	16/289	51.1 ± 15.6	22 (7.2)	5 (22.7)	16 (72.7)	1 (4.6)
Graves'	73	7/66	47.7 ± 14.4	2 (2.7)	0 (0.0)	2 (100)	0 (0.0)
Hashimoto's	232	9/223	52.1 ± 15.9	20 (8.6)	5 (25.0)	14 (70.0)	1 (5.0)

AITD: Autoinmune thyroid disease. Type of diabetes is indicated as follows: T1DM: type 1 diabetes mellitus, T2DM: non-insulin dependent type 2 diabetes mellitus, and IR-T2DM: insulin requiring type 2 diabetes mellitus.

in which our laboratory achieved the following scores: (i) 100% sensitivity, validity, and consistency for GADA in the third (1998) and fourth (1999) GADA Proficiency Test (Research Institute for Children, Harahan LA); (ii) 100% sensitivity, validity, specificity and consistency for IA-2A in the fourth (1999) IA-2A Proficiency Test (Research Institute for Children, Harahan LA); and (iii) 82% sensitivity, 82% validity, and 100% consistency and specificity for IAA (Immunology Diabetes Workshop, University of Florida, USA, 1998).

Cut-off values for GADA, IA-2A, IAA and PAA were calculated as means plus 3 SD of the control signals. For comparison purposes, results were expressed as precision units (SD scores). Comparisons between categorical data were made by Chi-square test with Yates' correction.

Results

Out of the 305 patients with autoimmune thyroid disease, 12 (3.9%) presented diabetes and were negative for all beta cell markers, while another group of 10 subjects (3.3%) were diabetic and positive for at least one marker.

TABLE 2.– *Association between autoimmune thyroid disease, clinical diabetes and anti beta-cell antibodies (Ab)*

	AITD n=305 (100%)	
	Diabetics n=22 (7.2%)	Non-diabetics n=283 (92.8%)
Ab (+)	n=10 (3.3%)	n=17 (5.6%)
	Total n=27 (8.9%)	
Ab (-)	n=12 (3.9%)	n=266 (87.2%)
	n=278 (91.1%)	

For details on the type of diabetes see the text and Tables 1 and 3. Ab: antibody.

TABLE 3.– *Clinical and immunological features of patients with antibodies to beta–cell proteins*

Patient	Sex	Age	Thyroid disease	Diabetic disease (*)	Anti β-cel markers (**)			
					GADA	IA-2A	IAA/IA	PAA
001	F	55	Hashimoto's	T1DM	**30.3**	0.3	**10.0** (***)	**16.9**
002	F	54	"	NO	**9.4**	-0.6	-2.0	-1.9
003	F	63	"	NO	**75.3**	**6.7**	-2.9	1.2
004	F	66	"	T2DM	1.2	**18.4**	-1.0	-0.8
005	F	76	"	NO	**15.5**	-0.7	-1.0	0.0
006	F	65	"	T2DM	**4.6**	0.8	0.0	0.6
007	M	36	"	NO	**3.1**	0.2	1.1	-0.2
008	F	59	"	NO	**44.5**	0.0	1.3	-1.2
009	F	47	"	T1DM	**44.4**	1.9	**30.9** (***)	**65.5**
010	F	76	"	NO	**101.5**	**6.0**	0.0	0.6
011	F	37	"	T1DM	**105.8**	1.4	**31.7** (***)	**50.5**
012	M	29	"	T1DM	2.3	**3.4**	2.3	**4.4**
013	F	53	"	NO	**65.8**	1.4	-0.6	-2.7
014	F	42	"	NO	**33.2**	1.2	0.9	-2.8
015	F	49	"	T2DM	**53.5**	1.3	0.3	-3.5
016	F	14	"	T1DM	**6.7**	**32.2**	**20.0** (***)	**55.0**
017	F	84	"	NO	**57.3**	1.6	-0.5	0.1
018	F	46	"	NO	**3.6**	2.3	-0.6	0.1
019	F	64	"	NO	**3.7**	1.7	-0.8	-1.1
020	F	73	"	IR-T2DM	**58.6**	2.3	**34.7** (***)	**55.7**
021	F	50	"	NO	**3.7**	1.7	-2.1	0.5
022	M	49	Graves'	NO	**16.2**	-0.7	-2.0	-0.2
023	F	42	"	NO	-1.0	-1.6	**5.3**	0.1
024	M	48	"	NO	**102.0**	0.9	0.2	-0.0
025	F	45	"	T2DM	**52.7**	1.2	-1.2	0.6
026	F	42	"	NO	**7.3**	1.9	-1.3	-2.5
027	F	37	"	NO	**30.5**	1.7	-2.5	0.6

Figures over cut off (bolded) were considered as positive. (*)Type of diabetes is indicated as follows: T1DM: type 1 diabetes mellitus, T2DM: adult-onset type 2 diabetes mellitus (when marker positivity was demonstrated, the actual classification must be considered as LADA), and IR-T2DM: insulin requiring type 2 diabetes mellitus with secondary failure to oral antidiabetic drugs. (**)Values are expressed as SD score. (***)In insulin treated patients the specific screened antibody was considered "insulin antibody" instead the conventional IAA marker.

Within the diabetic marker positive group, five subjects presented T1DM, four T2DM and one IR–T2DM. Other 17 patients (5.6%), although no diabetics, were positive for at least one marker. It is worth mentioning that whereas 10 of 22 (45.5%) patients with thyroid illness plus any variant of diabetes were marker-positive, 17 of 283 (6.0%) non-diabetic individuals were also positive for at least one marker (Tables 1 to 3).

At the time of diagnosis of thyroid disease, diabetes was present in 22 (7.2%) of the AITD patients. Twenty out of the 22 diabetic patients with AITD had Hashimoto's thyroiditis (8.6% of the total Hashimoto's patients), whereas only two were diagnosed as having Graves' disease (2.7% of the total Graves' patients) (Table 1).

Positive results, for at least one marker of autoimmune diabetes, were found in 27 of the 305 patients with AITD (8.9%), including 6 with Graves' and 21 with Hashimoto's disease, and only 10 of them (37.0%) associated to clinical diabetes. Twenty-four of the 27 AITD patients with beta-cell autoimmunity were positive for GADA, including 17 patients exclusively positive for GADA, 4 also positive for IAA and/or PAA, 2 also positive for IA-2A, and one positive for all the markers. From the remaining three GADA-negative patients, one with T1DM was positive for both PAA and IA-2A, one with T2DM was positive for IA-2A and one non-diabetic was positive for IAA (Table 3).

Clinical and immunological data of AITD patients with positive markers for beta-cell autoimmunity are detailed in Table 3. As part of the validation assessment, specificity of assays was checked and none of the 100 control samples tested for the four markers showed positive results.

Discussion

The association between T1DM and AITD has been known for a long time and there is also much evidence that T1DM may coexist with other autoimmune diseases[17-19]. Early evaluation of the risk of insulin dependent diabetes in AITD patients is likely to be important for successful therapeutic intervention, and detection of islet autoantibodies constitutes the main tool for such early diagnosis.

Autoantibodies to membrane-associated beta cell proteins, GADA and IA-2A, or to secreted products, as IAA and PAA, are usually detected before the onset of diabetes, and their presence is indicative of risk of developing the disease. The positive predictive value (PPV) for the presence of one or more markers in first-degree relatives of T1DM patients was evaluated in several studies. For example, in a large study in at risk individuals, the PPV at 5 years was 80% for IA-2A, 59% for IAA and 52% for GADA[20]. In addition, that work demonstrated that PPV increased with the number of different autoantibodies detected. Similarly, an increased risk of developing autoimmune diabetes was reported in patients with other endocrine autoimmune diseases[21]. The risk of developing diabetes in AITD patients has been also studied[3, 22], and some investigators were able to show that GADA positivity in non-diabetic individuals with autoimmune thyroiditis was associated with decreased maximal insulin secretory capacity and glucagon response to arginine[23]. These findings support the hypothesis that GADA is a marker for subclinical insulitis. In disagreement, Alsoy et al. were not able to demonstrate any effect of GADA positivity on either insulin secretion or insulin sensitivity parameters[24].

In early studies, the risk of developing autoimmune diabetes was evaluated through IAA and islet cell antibodies (ICA) determinations[25]. More recently, the overall sensitivity of marker detection has been increased by replacing ICA for the investigation of GADA, alone or combined with IA-2A by RBA using recombinant antigens[26-29].

In the present study, a large number of serum samples from patients with autoimmune thyroid disease were analyzed as a whole and after classifying them as individuals with Graves' or Hashimoto's disease. Overall, the studied population showed higher prevalence of autoimmune diabetes markers (8.9%) than the control population (0%) ($p<0.01$). Moreover, it is noteworthy that the prevalence of markers observed in Graves' and Hashimoto's disease was quite similar (8.2% and 11.6%, respectively) and no statistically significant difference was observed. Also, the prevalence of diabetes markers observed in Graves' and Hashimoto's population without clinical diabetes was similar in both groups (7.0% and 5.7%, respectively).

The most frequent marker in thyroid disease patients was GADA (7.2%) and similar values were observed comparing Graves' and Hashimoto's disease (6.8% and 8.2%, respectively). These results in our Argentine population agreed with previous studies on AITD from Maugendre et al.[22], Hallengren et al.[3] and Kawasaki et al.[30], who showed GADA prevalence ranging from 6.1 to 13.0%. These slightly different results can be attributed to the differences in study methods, population characteristics and sensitivity of the assays used for autoantibodies measurements. In the present work, GADA was the most prevalent marker (87.9%) among antibody-positive patients. From 17 non-diabetic marker-positive patients, 16 (94.1%) were GADA positive, while only two (11.8%) and one (5.9%) were IA-2A and IAA positive, respectively. This indicates that GADA determination is the best test for screening of individuals at risk of autoimmune-associated diabetes in the population with AITD. However, as stated by Maugendre et al.[21], the value of a single marker positivity for evaluating the risk of developing diabetes is doubtful. According to that work, patients with positivity only for GADA would present a lower frequency and an evident delay in the development of diabetes, than patients positive for more than one marker. In this sense,

some authors have observed that autoantibodies to IA-2 were strongly associated with acute onset of insulin dependent diabetes mellitus[31]. For instance, a large study on healthy relatives of diabetic patients revealed that PPV for diabetes at 3 and 5 years were 33% and 59%, respectively, in single-GADA positive individuals, but were 45% and 86%, respectively, in individuals with combined GADA and IA-2A positivity[20]. In the present transversal study, combined GADA and IA-2A positivity in AITD group was only 1.0%, suggesting that a longitudinal follow up of single positive marker in AITD patients may be a best protocol to assess the prodromic phase of associated autoimmune diabetes.

Finally, based on present results, we suggest including the routinely diabetes marker assessment in all AITD patients. In fact, 6.0% of diabetes marker prevalence here determined seems to be high enough to justify the early detection and hence the oportune therapeutic intervention of such patients probably evolutioning to insulin dependence. Moreover, the recent development of non-radiometric methods for GADA screening[32] is another reason to apply marker screening as part of a low cost/benefit diagnostic algorithm on patients sharing a common genetic background for autoimmune disease.

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Per molto che uno abbia letto, è ben difficile che al concepire un pensiero, lo creda suo, essendo d'altri; lo atribuisca all'inteletto, all'immaginazione propria, non appartenendo che alla memoria. Tali concezione sono accompagnate da certa sensazione che distingue le originali delle altre; e quel pensiero che porta seco la sensazione, per cosí dire, dell'originalità, verisimilissimamente non sarà stato mai concepito ugualmente da alcun altro, e sarà proprio e nuovo; dico, non quanto alla sostanza, ma quanto alla forma, che è tutto quel che si può pretendere. Giacchè è noto che la novità della più parte de 'pensieri degli autori più originali e pensatori consiste nella forma. (10 Maggio 1829).

Por mucho que uno haya leído, es bien difícil que al concebir un pensamiento, lo crea suyo, siendo de otro, lo atribuya al intelecto, a la imaginación propia, no perteneciendo sino a la memoria. Tales concepciones están acompañadas de cierta sensación que distingue las originales de las otras; y aquel pensamiento que lleva consigo la sensación, por así decirlo, de la originalidad, verosímilmente no será nunca concebido igualmente por algún otro, será propio y nuevo; digo, no en cuanto a la sustancia, sino en cuanto a la forma, que es todo aquello que se puede pretender, ya que es sabido que la novedad de la mayor parte de los pensamientos de los autores más originales y de los pensadores consiste en la forma.

Giacomo Leopardi (1798-1837)

Zibaldone di pensieri. Scelta a cura di Anna Maria Moroni, saggi introduttivi di Sergio Solmi e Giuseppe De Robertis (1937). (Oscar Clasicci). Milano: Mondadori, 1997, Volume II, pp 1180-1